

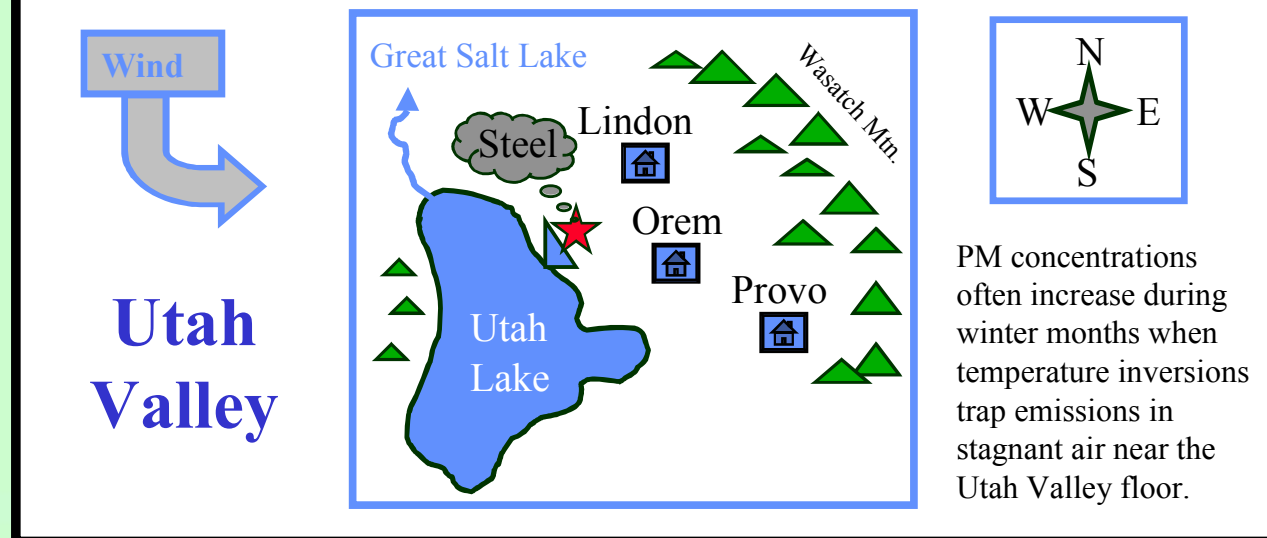
# Pulmonary Toxicity of Utah Valley PM: Are Empirical Indices of Adverse Health Effects Coherent with the Epidemiology?

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## Background

In 1989, Pope published a seminal article associating Utah Valley hospital respiratory admissions with PM<sub>10</sub> levels from April 1985 - February 1988, a period inclusive of intervals of closure and operation of the Geneva Steel Mill. While operational, this plant contributed  $\approx 82\%$  of all industrial-related PM in the Utah Valley (47-80% of all sources). On August 1, 1986 the steel mill closed due to a labor strike and remained closed for one year until reopening under new ownership on September 1, 1987. Ambient PM<sub>10</sub> measurements were made at a sampling site in Lindon, Utah downwind of the plant. Pope found that 83% of monthly respiratory hospital admissions for respiratory causes were significantly related to the mean as well as peak ambient PM<sub>10</sub> levels for both the immediate and previous months. Other admissions and time periods did not exhibit any correlation nor did hospitals in areas not affected by Geneva show correlations with PM<sub>10</sub>. These hospital admissions decreased soon after the plant closed and increased again when it reopened a year later as did public complaints of respiratory discomfort. The events of the closure and reopening of the steel mill provided a natural experiment for which morbidity among those in the affected area correlated, particularly among children, with lung or airway impairments (Pope CA, *Am J Pub Health* 1989; Pope CA, *Arch Env Health* 1991; Pope CA, *Toxicology* 1996).



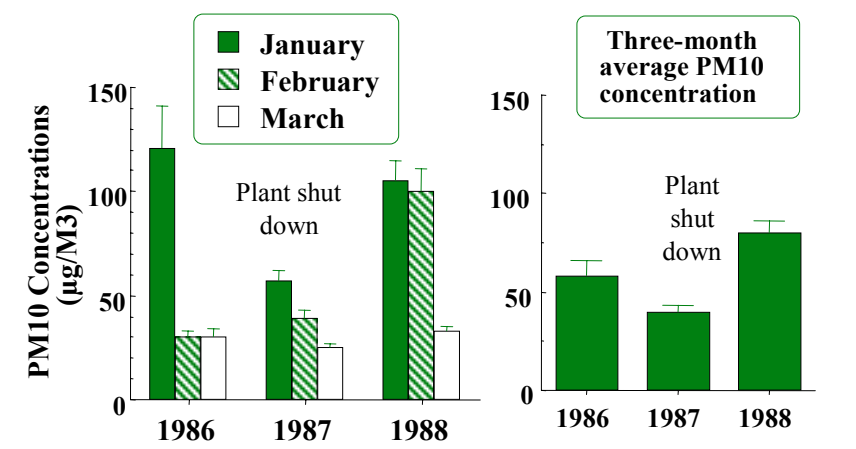
## Summary of Pope Findings for Utah Valley (1986 - 1988 Geneva Plant Operations)

- PM<sub>10</sub> correlations with hospital admissions coded for:
  - Simple pneumonia and pleurisy
  - Bronchitis and asthma
- Daily PM<sub>10</sub> > 150  $\mu\text{g}/\text{m}^3$  - children admissions increased 200%
- Daily PM<sub>10</sub> > 50  $\mu\text{g}/\text{m}^3$  - children admissions increased 100%
- Correlations found with concurrent and lagged PM concentrations
- Admissions patterned plant operations over 1986-1988



## Monitoring Data

Based on data from the AIRSData air pollution data base, monthly and 3-month winter PM<sub>10</sub> average concentrations at the Lindon, Utah monitoring site were lower during 1987.



## Study Purpose and Design

The National Research Council Report on "Research Priorities for Airborne Particulate Matter" has emphasized the need to establish biologic plausibility with regard to the health effects of PM as described by many recent epidemiological reports. Hence, the primary purpose of these studies was to enhance *biologic plausibility* of the link between the wintertime Utah Valley PM excursions and increased respiratory disease in the Utah Valley residents as was observed epidemiologically.

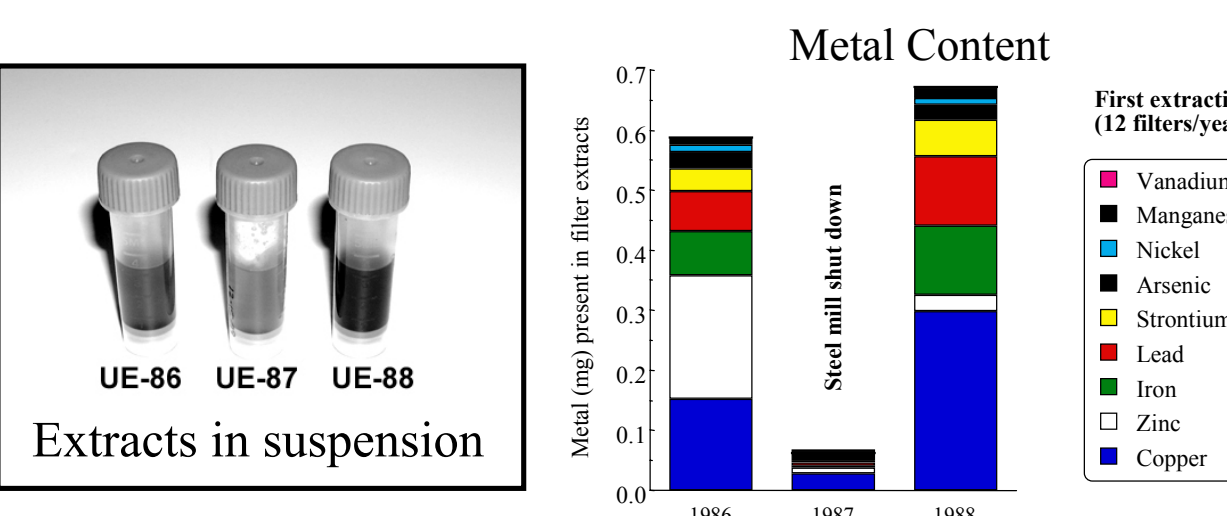
To this end, we obtained filters that were collected at the Lindon monitoring site for the winter months of the year before and during closure of the steel mill and the year after plant reopening. We extracted PM subcomponents from these filters. We characterized the water-based extracts in terms of their elemental composition, pH while in suspension, endotoxin content, and general solubility. Using equivalent masses of the extracts, we subsequently established their relative pulmonary toxicity using an integrated toxicological approach. *In vivo* studies were performed in both humans and laboratory animals. In addition, *in vitro* studies were performed utilizing pulmonary cell types from both humans and animals to better discern direct cellular and molecular effects of the extracts. As presented below, with this data base we attempted to establish whether or not the water-based extracts yielded toxicity data that was coherent with the aforementioned epidemiological observations.

## PM Extract Preparation and Analysis

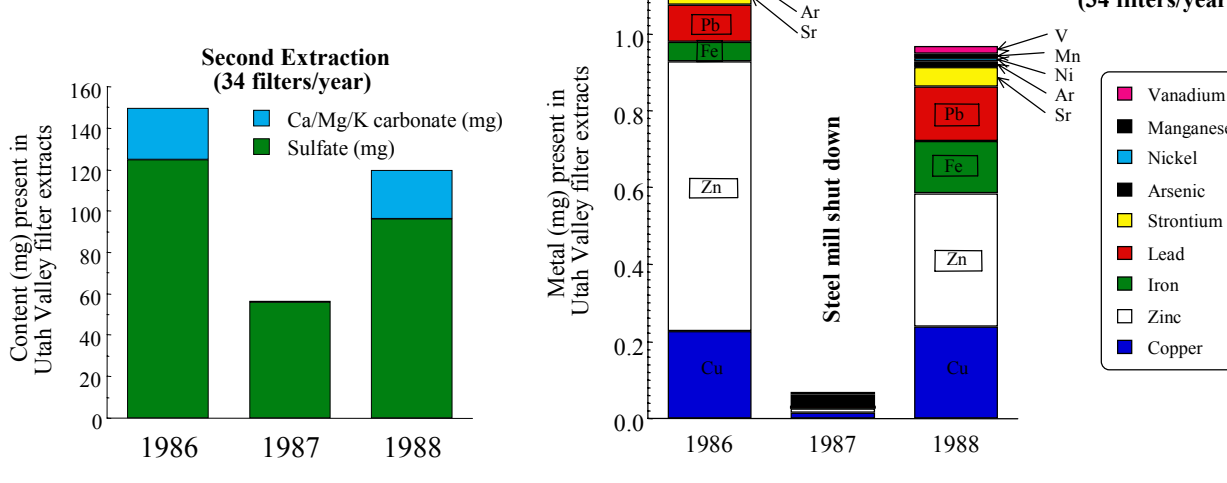
Archived hi-vol filters collected at the Lindon PM monitoring station in the Utah Valley were obtained from the Air Monitoring Center, Utah Division of Air Quality (Salt Lake City, UT). Equivalent numbers of filters from each of three consecutive winters were selected. The first winter defined as January-March of 1986 included filters collected prior to closure of the steel mill, filters from January - March of 1987 were collected during closure of the mill, and filters from January - March of 1988 were collected after the mill had reopened.

PM subcomponents were extracted from filters via agitation of filters in deionized water for 96 h. Filters were removed and the remaining liquid extracts were centrifuged to pellet relatively insoluble material. Supernatants from filters corresponding to 1986, 1987, and 1988 were pooled and lyophilized. Extracts were resuspended in 1.0 N HCl and analyzed for 40 different elements using inductively coupled plasma-mass spectroscopy (ICP-MS) closely following EPA method 6020 analytical protocol.

## Results of Utah PM Extract Analysis

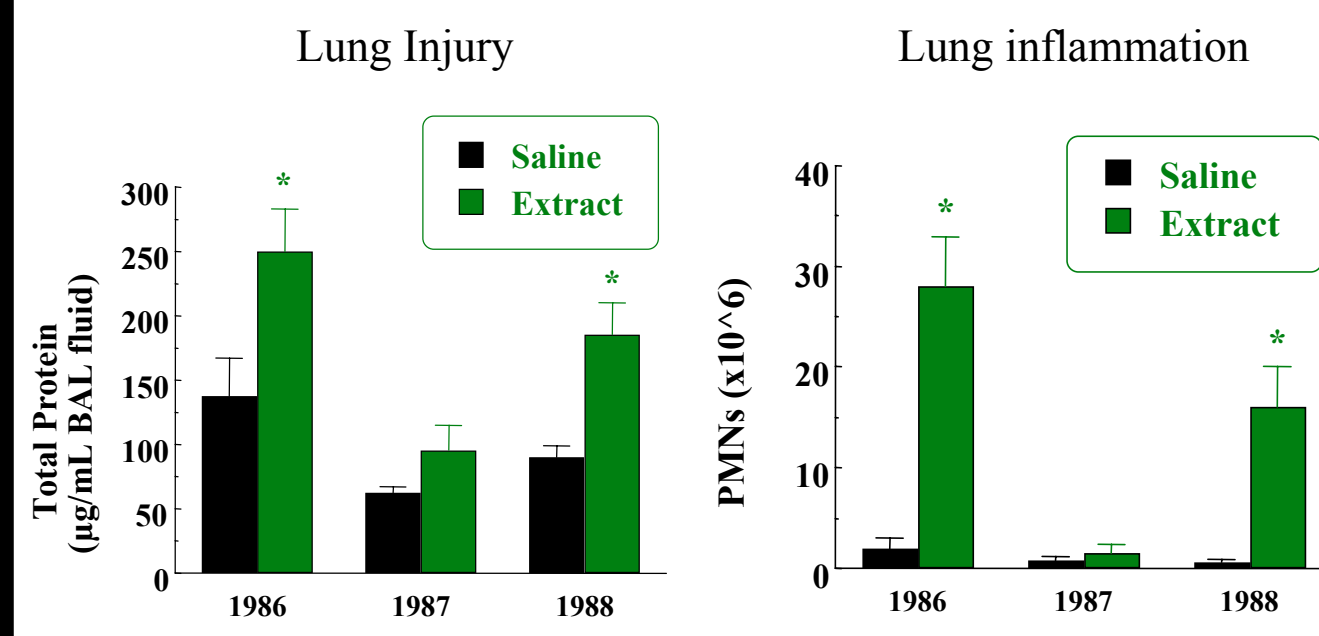


## Sulfate and Cation Content



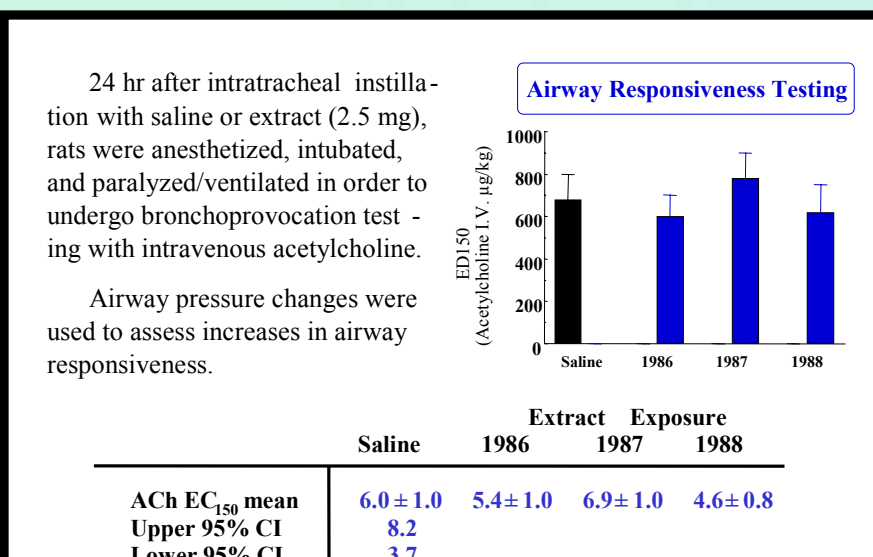
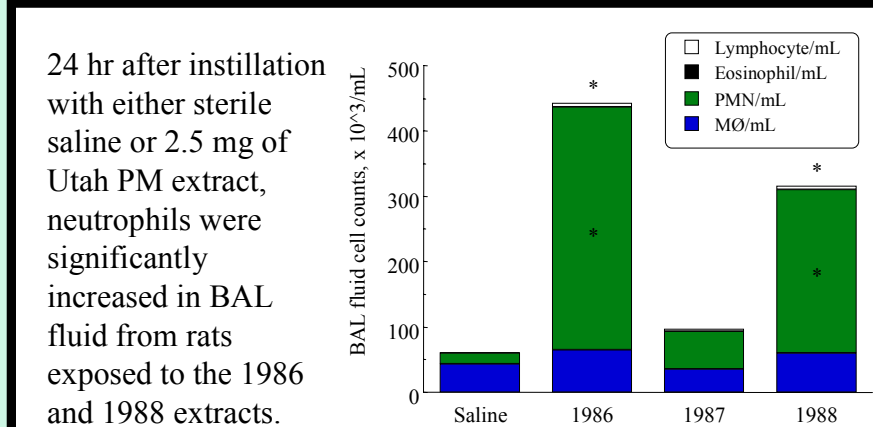
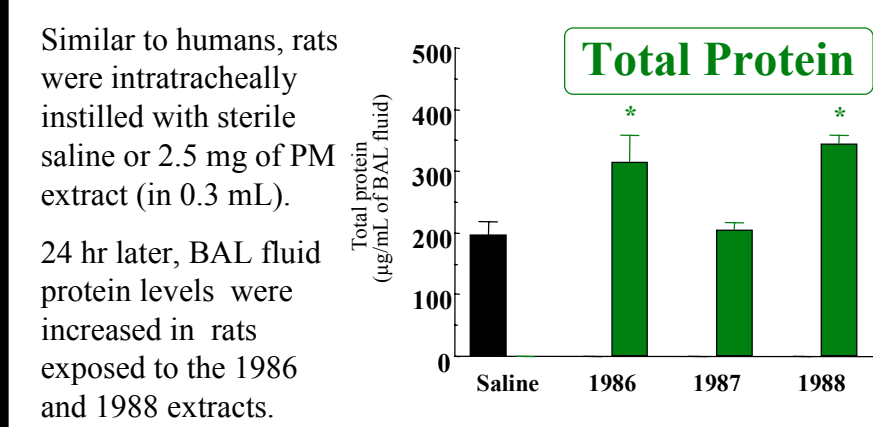
## In vivo Biological Responses

### Humans

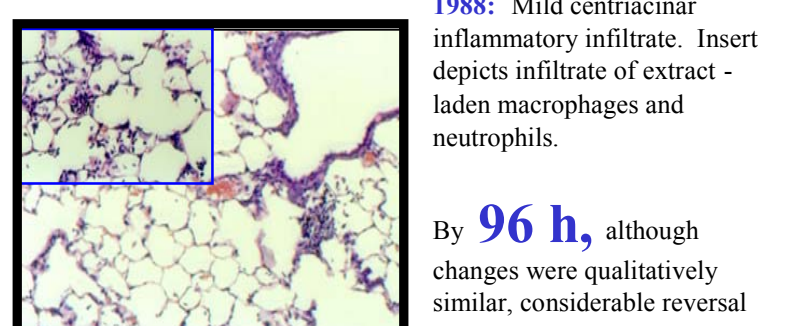
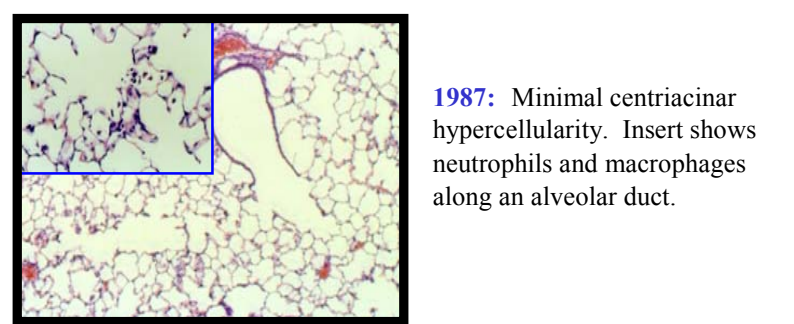
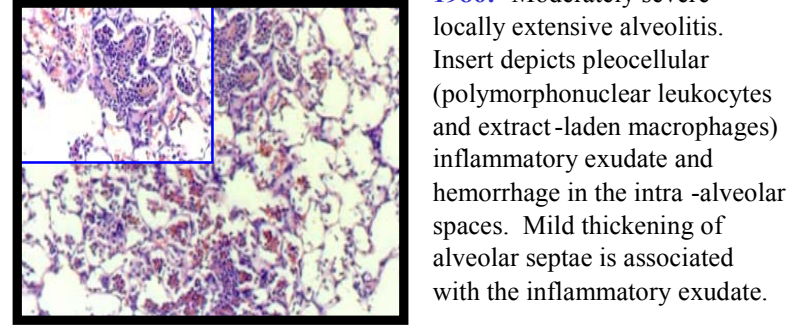
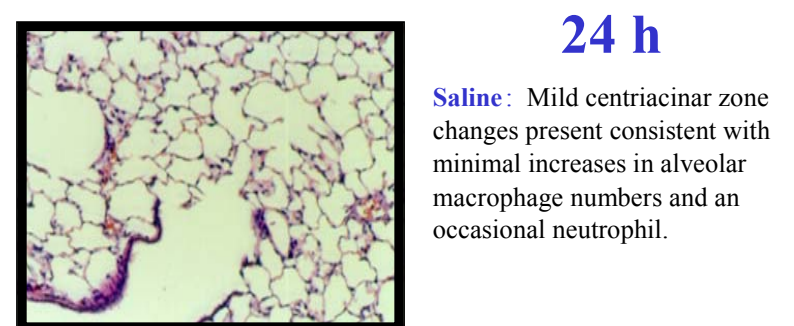


Humans were instilled via bronchoscopy. In contralateral lung lobes, sterile saline or 500  $\mu\text{g}$  of Utah Valley extract was instilled (in 20 mL). 24 hr later subjects underwent broncho-alveolar lavage (BAL) to assess changes in BAL fluid biochemical and cellular indices. Effects were observed after exposure to 1986 and 1988 (but not 1987) extracts. (Ghio AJ & Devlin RB, *Am J Respir Crit Care Med* 2001)

### Rodents



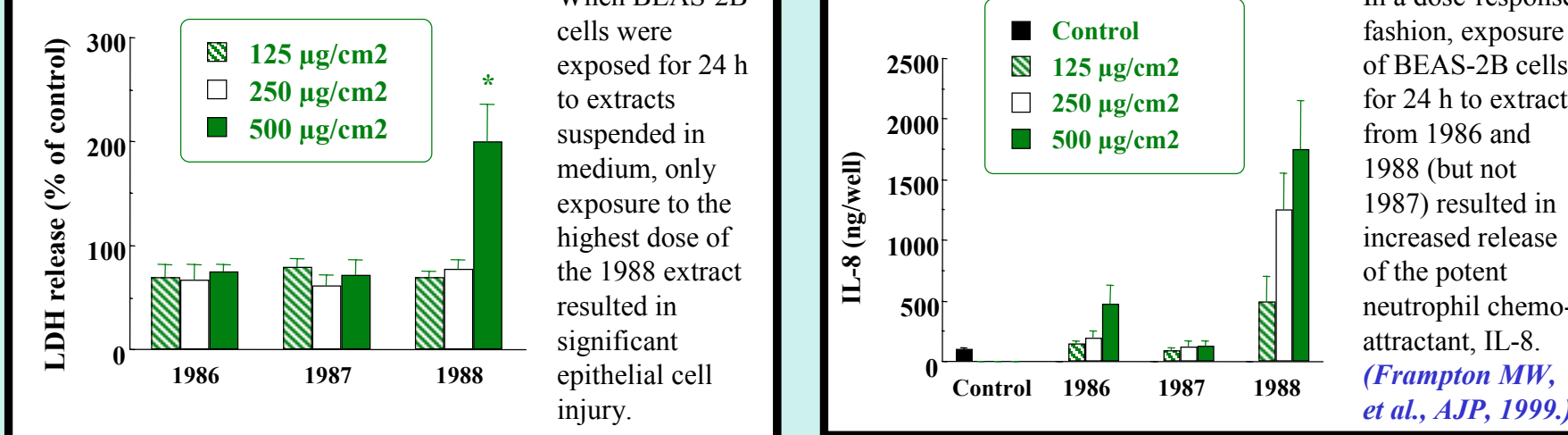
## Lung Pathology



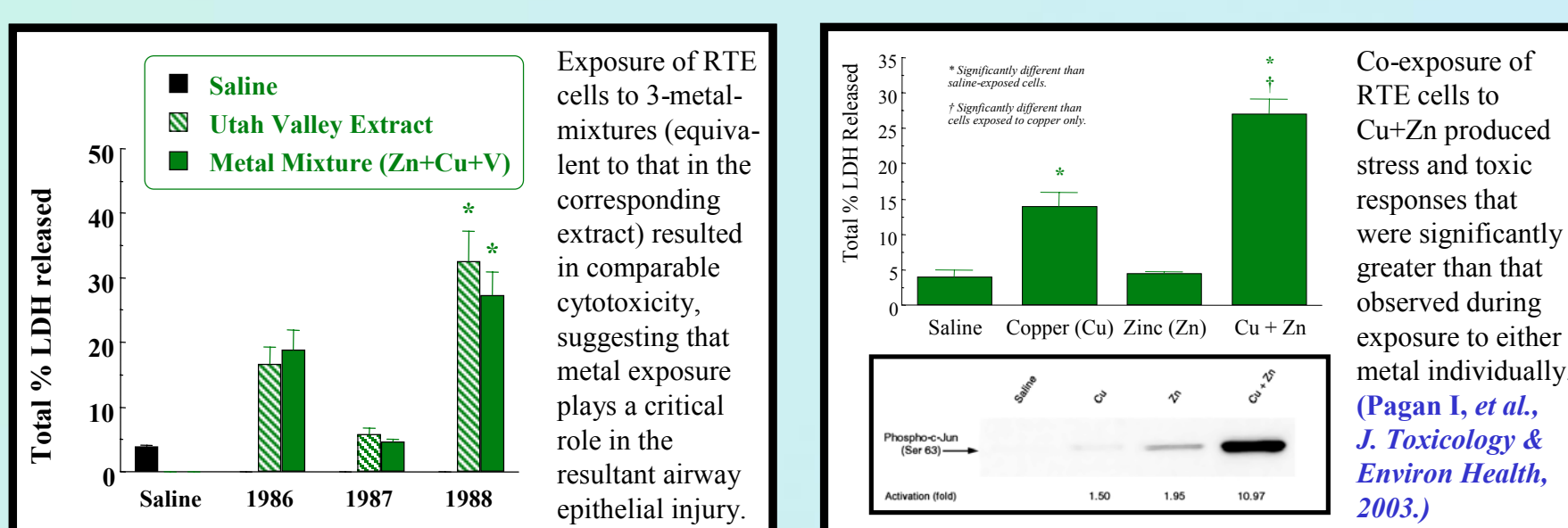
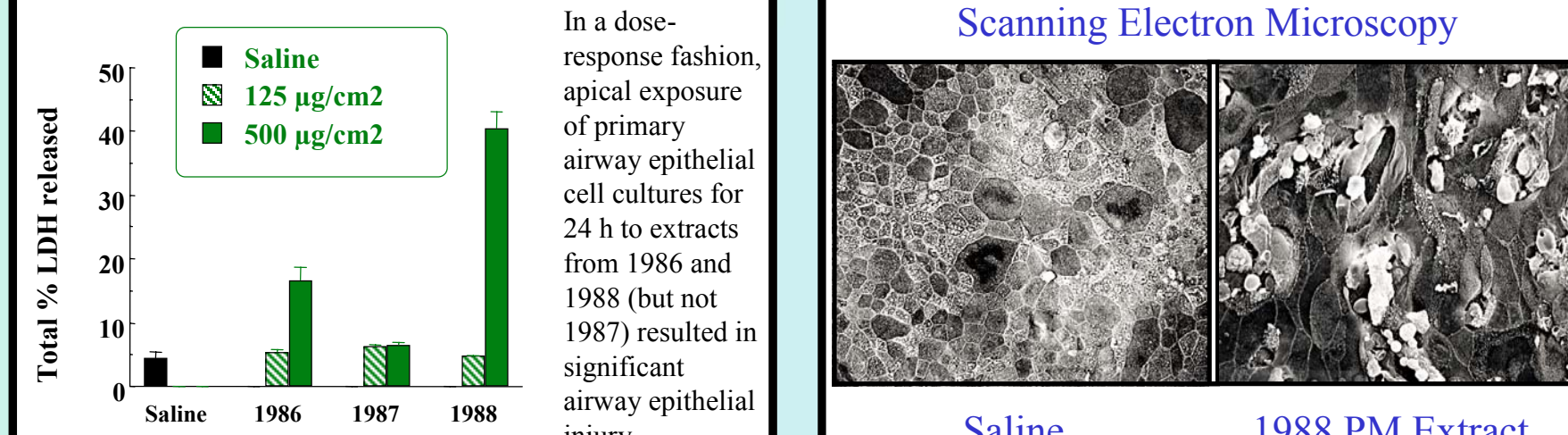
(Dye JA, et al., *Environ Health Perspectives* 2001)

## In vitro Biological Responses

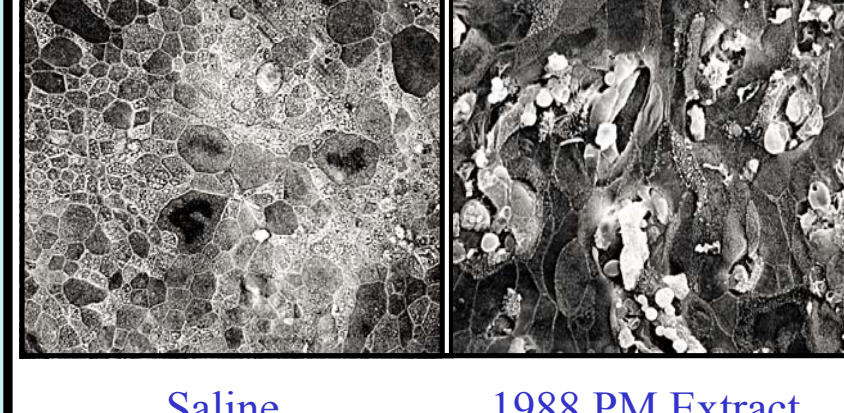
### Human airway epithelial (BEAS-2B) cells



### Rodent airway epithelial (RTE) cells



## Scanning Electron Microscopy



Saline 1988 PM Extract

## Mechanistic studies

### Effects on cell signaling pathways

- Activation of EGF receptor-dependent signaling in human airway epithelial cells exposed the Utah Valley PM. (Wu W et al., *Am J Physiology, Lung Cell Molecular Physiology*, 1999.) These mechanistic studies entailed exposing human airway epithelial cells in culture to Utah Valley extracts and determining the effects on phosphorylation/dephosphorylation of critical cell signaling pathways and on production of IL-8, a potent neutrophilic C-X-C chemokine.

### Effects on macrophages

- Soluble components of Utah Valley particulate pollution alter alveolar macrophage function *in vivo* and *in vitro*. (Soukup JM, Ghio AJ, and Becker S. *Inhalation Toxicology*, 2000.) These studies revealed that exposure of human alveolar macrophages to the 1986 extract resulted in an immediate oxidative response, decreased phagocytic function, and increased apoptosis. Exposure to 1986 and 1988 extracts inhibited macrophage oxidant activity. Such effects could lead to impaired pulmonary host defense mechanisms.

## Discussion

The operating status of the Geneva Steel plant between 1986 and 1988 provided an unusual opportunity to investigate observationally the potential link between PM and human health. Using an integrated toxicological approach, water-based extracts of local ambient PM filters from this same time period were used to assess the relative toxicity of ambient PM extracts from each of the three years. Data indicated that acute pulmonary injury, inflammation, and possibility pulmonary immune responses are consistently affected by exposure to extracts from 1986 and 1988, but not by exposure to the 1987 (plant off) extract. These effects have been demonstrated in both humans and animals using *in vivo* and *in vitro* methodologies. On the collective, these experimental findings are in good accord with the cross-sectional epidemiology studies.

## Impact

The ability to replicate in the laboratory what was determined from epidemiology studies has provided: (1) coherent and independent confirmation of the health effects findings, and (2) a "proof of concept" for the use of empirical experimental studies to assess and perhaps predict likely ambient PM adverse health effects. The collective use of these and analogous data from studies of ambient PM could thereby guide regulatory actions (NAAQS) based on refined risk assessments, and perhaps specifically proximate toxicants beyond PM - mass metric that might warrant specified emission control changes.

## Future Directions

The conceptual linkage between studies of human populations and empirical laboratory studies validates the further use of controlled human and animal studies that are designed to define underlying mechanisms to refine risk assessment paradigms and regulatory actions. We have initiated plans to collaborate with Supersite investigators to devise empirical assessments of not only components of PM and related cofactors, but to use detailed site-specific source apportionment models to ascertain source contributions to PM-associated toxicity.

Results emanating from these studies will refine our approach to investigating the role of metals and various PM copollutants in cardiopulmonary health effects of PM. Where possible larger quantities of ambient PM will be collected to elaborate upon the Utah Valley data base. The current broad strategy mirrors that used in the Utah Valley project, which includes *in vivo* human and animal studies with a strong effort to elucidate mechanisms of injury at the organ, cellular, and molecular level. Important in the evolution of the program is the cautious use of human subjects with disease as well as the considered characterization and use of animal and *in vitro* cell models that mimic analogous human disease conditions that appear to contribute to susceptibility. The strengths imparted by an integrated program of epidemiological, human, animal, and *in vitro* studies can only augment the quantitative risk evaluations and regulatory decision-making.

## Acknowledgements

The authors also recognize the scientific contributions of Dr. M. W. Frampton (University of Rochester, Rochester, NY), Dr. J. I. Everitt (CHT Centers for Health Research, RTP, NC), and Dr. M. K. Dykstra (North Carolina State University, Raleigh, NC) on these investigations. We also thank Mr. G. Ross (SEE Program, RTP, NC) and the personnel at the Air Monitoring Center, Utah Division of Air Quality, Salt Lake City, Utah.

## Level 1 STAA Award (2002)

Title: Air pollution particles from the Utah Valley cause lung injury and inflammation in humans and animals.

Contributing Papers:

- Frampton, M. W., A. J. Ghio, J. M. Samet, J. L. Carson, J. D. Carter and R. B. Devlin (1999). "Effects of aqueous extracts of PM<sub>10</sub> filters from the Utah valley on human airway epithelial cells." *Am J Physiol* 277(5 Pt 1): L960-967.
- Soukup, J. M., A. J. Ghio and S. Becker (2000). "Soluble components of Utah Valley particulate pollution alter alveolar macrophage function *in vivo* and *in vitro*." *Inhalation Toxicol* 12(5): 401-414.
- Dye, J. A., J. R. Lehmann, J. K. McGee, D. W. Winsett, A. D. Ledbetter, J. I. Everitt, A. J. Ghio and D. L. Costa (2001). "Acute pulmonary toxicity of particulate matter (PM) filter extracts in rats: Coherence with epidemiological studies in Utah Valley residents." *Environ Health Perspect* 109 (Sup 3): 395-403.
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- Wu, W., J. M. Samet, A. J. Ghio, and R. B. Devlin (2001). "Activation of the EGF receptor signaling pathway in airway epithelial cells exposed to Utah Valley PM." *Am J Physiol Lung Cell Mol Physiol* 281(2): L483-L489.
- Pagan, I., D. L. Costa, J. K. McGee, J. H. Richards, M. J. Dykstra and J. A. Dye (2003). "Metals mimic airway epithelial injury induced by *in vitro* exposure to Utah Valley ambient particulate matter extracts." *J Toxicol Environ Health*, Part A, 66(12):126.